

AMENDMENTS TO THE SPECIFICATION

Amendments to be made to the specification are indicated below.

Please amend paragraph [0018], as follows:

[0018] In some examples, the porcine adenovirus sequence essential for encapsidation is a porcine adenovirus 3 sequence. In some examples, the porcine adenovirus sequence essential for encapsidation of porcine adenovirus type-3 is located between about nucleotide position 212 and about nucleotide position 531 (SEQ ID NO:414) at the left end of the genome. In other examples, the porcine adenovirus sequence essential for encapsidation is a porcine adenovirus 5 sequence. In other examples, an isolated porcine adenovirus sequence essential for encapsidation comprises a nucleotide sequence selected from the group consisting of:

CGGAAATTCCCGCACA; GGCAGAAATTCCCGCACA;
GGGATTTGTGCCCTCT; GCGGGATTTGTGCCCTCT
CGGTATTCCCCACCTG; CCCGGTATTCCCCACCTG
GTGTATTTTCCCCTCA; GGGTGTATTTTCCCCTCA
GTGTATATAGTCCGCGC; CAGTGTATATAGTCCGCGC;
GAGTTTCTCTCAGCG; and TAGAGTTTCTCTCAGCG.

Please amend paragraph [0054], as follows:

[0054] Data shown herein demonstrate that the packaging motifs (sequences) of PAV3 are located between nucleotides 212 and 531 (SEQ ID NO:414) on the left end of the viral genome. There are at least six AT-rich motifs which can provide the packaging ability to PAV3. They overlap the promoter region of E1A gene of the virus. Packaging motifs of PAV3 have an AT-rich sequence followed by a GC-rich region. They appear to be functionally redundant, that is one or more encapsidation sequences can be deleted and viral DNA can still be encapsidated. Some of them alone can support the viral packaging and make PAV viable. The present invention also

encompasses PAV sequence(s) essential for encapsidation of other PAVs such as for example, PAV5.

Please amend paragraph [0055], as follows:

[0055] Transcriptional control regions of PAV E1 have been defined and are disclosed herein. The packaging domain between nucleotides 212 and 531 (SEQ ID NO:414) overlap with the transcriptional control region of E1. The present invention provides novel adenovirus vectors and adenovirus comprising modifications in the E1 transcriptional control regions disclosed herein.

Please amend paragraph [0079], as follows:

[0079] The present invention relates to the identification and characterization of PAV regions essential for encapsidation, also referred to herein as packaging domains. Based on the identification of cis-acting packaging domain of human adenovirus 5 (HAV5), 5' TTTGN8CG-3' (Schmid et al. 1997, *J. Virol.* 71:3375-3384) the PAV3 genome was searched to identify putative packaging domains. The packaging domain of porcine adenovirus type-3 is located between about nucleotide position 212 and about 531 (SEQ ID NO:414) at the left end of the genome. No regions were found that showed perfect homology with the consensus packaging domain of HAV5. As shown in the examples, a series of mutations were made in PAV-3 genome in order to determine the regions essential for PAV encapsidation. Data shown herein in the examples demonstrate that there are at least six AT-rich motifs which can provide the packaging ability to PAV3. Table 1 provides a listing of the regions.

Please amend paragraph [0091], as follows:

[0091] By analyzing porcine adenovirus type 3 (PAV-3) mutants containing deletion mutations in transcriptional control region of E1A transcription unit, a functionally two-faced regulatory element located upstream of TATA box of E1A promoter was defined. The E1 transcriptional control region overlaps the packaging domain between nucleotides(nt) 212 and 531 (SEQ ID NO:414) and is demonstrated herein to be between about nucleotide 212 to about

nucleotide 449. All E1 transcriptional control region nucleotide numbering is based on the PAV3 nucleotide sequence disclosed in Reddy et al. 1998, *Virology* 251:414-426 and the sequence of PAV3 nucleotides 371 to 490 is disclosed in Figure 13B.

Please amend paragraph [00101], as follows:

[00101] As disclosed herein, the PAV-3 cis-acting packaging domain is located between about nucleotides 212 and 531 (SEQ ID NO:414). A packaging domain is required for encapsidation of PAV-3 DNA into virions late in the viral life cycle. Packaging domains overlap the transcriptional control region of E1A and consists of at least six AT-rich units with functional redundancy and importance hierarchy.

Please amend paragraph [0164], as follows:

[0164] Another set of virus mutants contain deletions which progress from a common site at nt 531 towards the upstream border of the packaging domain (Figure 8 and 9). When a deletion was located between nt 212 and 531 (SEQ ID NO:414), virus was not obtained. The results suggested that the packaging domain of PAV3 probably existed between nt 212 and 531 (SEQ ID NO:414). The existence of DNA sequences between nt 212 and 252 made the virus viable and suggested that there should be a packaging motif in this region, which was probably the upstream border packaging motif of PAV3. The PAV3-252/531 showed a lower level of growth in VIDO R1 cells in single infection and a lower packaging ability in coinfection when compared with mutant PAV3-151/497 (Figure 6). The result showed that the downstream border packaging motif between nt 474 and 497 can provide the stronger packaging ability to PAV3 than the upstream border packaging motif between nt 212 and 252. From PAV3-252/531 to PAV3-382/531, the sequential addition of DNA sequences between nt 252 and 382 resulted in the increase in both the viral growth level and packaging ability. Results suggested that there would be two packaging motifs between nt 252 and 382. Pav3-382/531 has the same growth property and packaging ability as compared with PAV3-432/531. The addition of DNA sequences between nt 382 and 432 has no effect on the viral packaging. The further addition of DNA sequences between nt 432 and 447 made both the viral

growth and packaging ability increase. This suggested that the AT-rich motif between nt 432 and 447 could function as a packaging motif.

Please amend paragraph [0167], as follows:

[0167] In conclusion, the packaging domain of PAV3 is located between nt 212 and 531 (SEQ ID NO:414) on the left end of viral genome. There are at least six AT-rich motifs which can provide the packaging ability to PAV3. They overlap the promoter region of E1A gene of the virus. Packaging motifs of PAV3 have an AT-rich sequence followed by GC-rich region. They are functionally redundant and show an importance hierarchy. Some of them alone can support the viral packaging and make virus viable.

Please amend paragraph [0185], as follows:

[0185] The PAV-3 cis-acting packaging domain is located between nt 212 and 531 (SEQ ID NO:414) and overlaps the transcriptional control region of E1A. Because packaging efficiency directly determines the production of infectious viral progeny, the growth phenotypes of mutant viruses might be complicated by packaging defect. To exclude the effects of deletion of cis-acting packaging motif(s) on the viral growth, DNA accumulation in virus-infected cells was examined by Southern blot analysis. ST cells were infected with wild-type or mutant virus at a MOI of 5 PFU per cell. At 9, 16, 23 and 30 h postinfection, the cells were collected and high-molecular DNA was isolated. After digestion with *Hind*III, DNA fragments were separated by agarose gel electrophoresis and subjected to Southern hybridization. Representative results are shown in Fig. 17 Pav1615 (nt 447-474) and Pav516 (nt 382-474) displayed the defective DNA accumulation throughout the infection (16, 23 and 30h postinfection). The results are in good agreement with their growth properties (Fig. 16). Pav59 (nt 382-433) carrying the deletion of regulatory element showed the similar rate of DNA accumulation at the late times (23 and 30h) postinfection, but displayed a lower rate of DNA accumulation at the early time (16h) postinfection compared with that of wild-type virus. However, Pav1413 (nt 432-449), Pav514 (nt 382-449), and wild-type PAV-3 showed the similar rate of DNA accumulation. These results suggested that the regulatory

element appears to affect the onset of DNA replication. Surprisingly, Pav16 (nt 151-254) grew to titers 3 fold less than wild-type PAV-3. In addition, Pav16 showed a lower rate of DNA accumulation at early (16h) and late times (23 and 30 h) postinfection.